

Claims

1. A method for producing a heritable integration of a transgene within a genome of a somatic or germ line cell of an invertebrate organism, the method comprising:
 - providing a first DNA cassette within said genome, wherein said first cassette comprises a first flanking transposon half side, a second flanking transposon half side, and an internal transposon half side, wherein said internal transposon half side and said first flanking transposon half side form a pair of excisable transposon half-sides, and wherein said first cassette further comprises said transgene in-between the internal transposon half side and said second flanking transposon half side; and
 - mobilizing said excisable transposon half-sides.
2. The method of claim 1, wherein said internal transposon half side and said second flanking transposon half side are TransposonL half sides, and wherein said first flanking transposon half side is a TransposonR half side.
3. The method of claim 1, wherein said internal transposon half side and said second flanking transposon half side are TransposonR half sides, and wherein said first flanking transposon half side is a TransposonL half side.
4. The method of claim 1, wherein said excisable transposon half-sides and corresponding transposase enzyme are from a transposable element, wherein said transposable element has terminal inverted sequences, and wherein said transposable element transposes via a DNA-mediated process.
5. The method of claim 1, wherein said first DNA cassette further comprises a first selectable marker gene located between said internal transposon half side and said first flanking transposon half side, and a second selectable marker gene located between said internal transposon half side and said second flanking transposon half side, and wherein said first and second selectable marker genes are phenotypically distinguishable.
6. The method of claim 5, wherein said first and second marker genes are, in either order,

any combination of marker genes producing distinguishable fluorescent or other visible dominant phenotypes.

7. The method of claim 5 wherein said first and second marker genes are, in either order, a combination of the transformation marker genes PUbDsRed1 and 3xP3-ECFP.
8. The method of claim 1, wherein said internal transposon half side is provided in reverse orientation, wherein said excisable transposon is formed by inversion of said internal transposon half side relative to said first flanking transposon half side, wherein said internal transposon half side further comprises flanking recombinase sites, and wherein said inversion is catalyzed by a site-specific recombinase.
9. The method of claim 8, wherein said recombinase sites are FRT sites in opposite or reverse orientation.
10. The method of claim 1, wherein said excisable transposon is mobilized by a source of transposase corresponding to said excisable transposon to render the remaining genomic DNA immobilizable.
11. A method for targeting a heritable integration of a transgene within a genome of a somatic or germ line cell of an invertebrate organism, said method comprising:
integrating a first DNA cassette within said genome by transposase-mediated integration of flanking transposon half sides, wherein said first cassette comprises a wild-type/non-mutated or a mutated target site of a site-specific recombinase at one end and a mutated target site of said site-specific recombinase at an other end, wherein said recombinase target sites are heterospecific, and wherein said target sites flank marker gene DNA and additional DNA sequence, and
exchanging said first DNA cassette for a second DNA cassette by a site-specific recombinase enzyme that catalyzes a DNA recombination reaction via homospecific recombinase target sites.
12. The method of claim 11, wherein said site-specific recombinase is FLP recombinase,

- and wherein said recombinase target sites are FRT sites or mutated derivatives of said FRT sites.
13. The method of claim 11, wherein said site-specific recombinase is Cre recombinase, and wherein said recombinase target sites are loxP sites or mutated derivatives of said loxP sites.
14. The method of claim 11, wherein said first cassette comprises one site-specific recombinase target site placed in-between a marker gene coding region and a promoter DNA that regulates its expression.
15. The method of claim 11, wherein said first cassette comprises a homing sequence to enhance pairing to said site-specific recombinase target sites in said second cassette.
16. The method of claim 11, wherein said homing sequence comprises a DNA sequence hybridizing to a *Drosophila linotte* locus.
17. The method of claim 11, wherein said second cassette comprises said heterospecific site-specific recombinase target sites.
18. The method of claim 17, wherein said second cassette comprises a marker gene coding region lacking a promoter for regulating its expression, and wherein, following the exchange of said first DNA cassette to said second cassette, said marker gene is placed under the control of said promoter derived from said first cassette.
19. The method of claim 17, wherein said second cassette comprises the same homing sequence as said first cassette within said recombinase target sites.
20. The method of claim 17, wherein said second cassette has a transposon half side in-between said recombinase target sites with phenotypically distinguishable marker genes on either side, wherein one of said marker genes lacks a promoter.

21. The method of claim 17, wherein site-specific recombinase mediated insertion occurs between a coding region of said second cassette and an operable promoter of a selectable marker gene of said first cassette.
22. The method of claim 20, wherein said internal transposon half side is excisable with a flanking transposon half side, and wherein said excisable transposon is mobilized by a source of transposase corresponding to said excisable transposon to render the remaining genomic DNA immobilizable.
23. An invertebrate organism comprising the heritable transgene produced according to claim 1.
24. An invertebrate organism comprising the heritable transgene produced according to claim 11.
25. A method for producing a heritable integration of a transgene within a genome of a somatic or germ line cell of an organism, the method comprising:

providing a first DNA cassette within said genome, wherein said first cassette comprises a first flanking transposon half side, a second flanking transposon half side, and an internal transposon half side, wherein said internal transposon half side and said first flanking transposon half side form a pair of excisable transposon half-sides, and wherein said first cassette further comprises said transgene in-between the internal transposon half side and said second flanking transposon half side; and

mobilizing said excisable transposon half-sides.
26. The method of claim 25, wherein said internal transposon half side and said second flanking transposon half side are TransposonL half sides, and wherein said first flanking transposon half side is a TransposonR half side.
27. The method of claim 25, wherein said internal transposon half side and said second flanking transposon half side are TransposonR half sides, and wherein said first flanking transposon half side is a TransposonL half side.

28. The method of claim 25, wherein said excisable transposon half-sides and corresponding transposase enzyme are from a transposable element, wherein said transposable element has terminal inverted sequences, and wherein said transposable element transposes via a DNA-mediated process.
29. The method of claim 25, wherein said first DNA cassette further comprises a first selectable marker gene located between said internal transposon half side and said first flanking transposon half side, and a second selectable marker gene located between said internal transposon half side and said second flanking transposon half side, and wherein said first and second selectable marker genes are phenotypically distinguishable.
30. The method of claim 29, wherein said first and second marker genes are, in either order, any combination of marker genes producing distinguishable fluorescent or other visible dominant phenotypes.
31. The method of claim 29, wherein said first and second marker genes are, in either order, a combination of the transformation marker genes PUbDsRed1 and 3xP3-ECFP.
32. The method of claim 25, wherein said internal transposon half side is provided in reverse orientation, wherein said excisable transposon is formed by inversion of said internal transposon half side relative to said first flanking transposon half side, wherein said internal transposon half side further comprises flanking recombinase sites, and wherein said inversion is catalyzed by a site-specific recombinase.
33. The method of claim 32, wherein said recombinase sites are FRT sites in opposite or reverse orientation.
34. The method of claim 25, wherein said excisable transposon is mobilized by a source of transposase corresponding to said excisable transposon to render the remaining genomic DNA immobilizable.

35. A method for targeting a heritable integration of a transgene within a genome of a somatic or germ line cell of an organism, said method comprising:

integrating a first DNA cassette within said genome by transposase-mediated integration of flanking transposon half sides, wherein said first cassette comprises a wild-type/non-mutated or a mutated target site of a site-specific recombinase at one end and a mutated target site of said site-specific recombinase at an other end, wherein said recombinase target sites are heterospecific, and wherein said target sites flank marker gene DNA and additional DNA sequence, and

exchanging said first DNA cassette for a second DNA cassette by a site-specific recombinase enzyme that catalyzes a DNA recombination reaction via a homospecific recombinase target site.

36. The method of claim 35, wherein said site-specific recombinase is FLP recombinase, and wherein said recombinase target sites are FRT sites or mutated derivatives of said FRT sites.

37. The method of claim 39, wherein said site-specific recombinase is Cre recombinase, and wherein said recombinase target sites are loxP sites or mutated derivatives of said loxP sites.

38. The method of claim 35, wherein said first cassette comprises one site-specific recombinase target site placed in-between a marker gene coding region and a promoter DNA that regulates its expression.

39. The method of claim 35, wherein said first cassette comprises a homing sequence to enhance pairing to said site-specific recombinase target sites in said second cassette.

40. The method of claim 35, wherein said homing sequence comprises a DNA sequence hybridizing to a *Drosophila linotte* locus.

41. The method of claim 35, wherein said second cassette comprises said heterospecific

site-specific recombinase target sites.

42. The method of claim 41, wherein said second cassette comprises a marker gene coding region lacking a promoter for regulating its expression, and wherein, following the exchange of said first DNA cassette to said second cassette, said marker gene is placed under the control of said promoter derived from said first cassette.
43. The method of claim 41, wherein said second cassette comprises the same homing sequence as said first cassette within said recombinase target sites.
44. The method of claim 41, wherein said second cassette has a transposon half side intervening between said recombinase target sites with phenotypically distinguishable marker genes on either side, wherein one of said marker genes lacks a promoter.
45. The method of claim 41, wherein site-specific recombinase mediated insertion occurs between a coding region of said second cassette and an operable promoter of a selectable marker gene of said first cassette.
46. The method of claim 44, wherein said internal transposon half side is excisable with a flanking transposon half side, and wherein said excisable transposon is mobilized by a source of transposase corresponding to said excisable transposon to render the remaining genomic DNA immobilizable.
47. An organism comprising the heritable transgene produced according to claim 25.
48. An organism comprising the heritable transgene produced according to claim 35.